Discriminative behavioral responses by aphids to various plant matrix polysaccharides

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Abstract

Three species of aphids, *Schizaphis graminum* (Rondani) (the greenbug), *Acyrthosiphon pisum* (Harris) (the pea aphid) and *Myzus persicae* (Sulzer) (the green peach aphid), showed significantly different behavioral responses to various plant mono- or polysaccharides incorporated within thin (<200 μm) films of agar overlaying artificial diets. Thirteen polysaccharides (i.e.: polygalacturonic acid, low methoxy pectin, high methoxy pectin, arabinogalactan, xylan, galactan, cellulose, starch, amylose, laminarin, dextran, 2,3-diacetyl pectin and trifluoroacetyl pectin) and 5 monosaccharides (arabinose, xylose, galactose, glucose and galacturonic acid) were tested in paired choices against a control overlay containing only agar. Glucose, dextran and laminarin were the only compounds that all three species of aphids responded to similarly (viz., glucose stimulated, dextran had no effect and laminarin deterred ingestion of underlying diets). None of the aphids exhibited negative responses to the monosaccharides tested. These monosaccharides either had no significant effect or were stimulatory. The polysaccharides tested induced a variation of unaltered, stimulatory or inhibitory behavioral responses in the 3 species of aphids. The behavioral responses of both greenbugs and pea aphids, which are oligophagous, were significantly affected by 6 out of 7 plant matrix polysaccharides tested. Feeding behavior by green peach aphids was affected by only one of these polysaccharides.

Electronic monitoring of aphid probing showed that aphids salivated in and 'test' probed the overlays prior to inserting their stylets into and ingesting from the underlying diets. Recorded responses of aphids probing overlays containing inhibitory polysaccharides resembled the recorded behavioral responses of aphids on resistant or nonhost plants (i.e., numerous short probes with either a lack of or a prolonged period prior to ingestion). The differential behavioral responses of the aphids to the various polysaccharides supports the view that plant matrix polysaccharides play a role in the chemical basis of aphid – plant interactions.

Introduction

The chemical basis of plant-aphid interactions is frequently attributed either to plant secondary compounds (e.g., phenolics, alkaloids, etc.) or to variations in levels of nutrients that are requisite for normal aphid development. Various phenolics (Dreyer & Jones, 1981; references therein) and alkaloids (Corcuera, 1984; Schoonhoven & Derksen-Koppers, 1976), when extracted from plants and incorporated into artificial diets, can significantly affect aphid feeding behavior. However, these types of plant compounds (i.e., allelochemicals) are generally compartmentalized in vacuoles of mesophyll cells (Matile, 1984). In the course of stylet penetration towards the phloem, aphids are able to minimize the intake of such deleterious plant compounds by withdrawing their stylets from and/or not ingesting the contents of mesophyll cells or their vacuoles. Support for the diminished intake of plant allelochemicals by aphids is the low *trans*-epoxide hydrolase activity in

a polyphagous aphid, *M. persicae*. This enzyme is believed to play a role in the detoxification of plant allelochemicals and herbivorous, chewing insects exhibit relatively high *trans*-epoxide hydrolase activity (Mullin & Croft, 1984). A nutritional basis of aphid-plant interactions is equivocal. Aphid fecundity and population growth are not always positively correlated with higher foliar levels of nitrogen or amino acids when different varieties of plants are considered (van Emden & Bashford, 1976). Similarly, foliar levels of some other essential insect nutrients do not necessarily correlate with aphid fecundity on different plant varieties (phytosterols: Campbell & Nes, 1983; cyclitols: Campbell & Binder, 1984).

It was recently asserted that plant matrix polysaccharides play a role in aphid-plant interactions. Aphids which probe plant tissues intercellularly possess salivary pectinases (Adams & McAllen, 1958) and other carbohydrases capable of degrading other plant matrix polysaccharides, such as cellulose and hemicelluloses (Adams & Drew, 1965; Campbell & Dreyer, 1985). The role of these polysaccharide degrading enzymes in the saliva of aphids is presumed to facilitate aphid stylet penetration of plant tissues (Pollard, 1973). Consistent with this viewpoint is the observation that resistance of sorghum towards an aphid-pest, the greenbug (*S. graminum*), was associated with the reduced ability of greenbug polysaccharases to depolymerize sorghum pectic substances from resistant cultivars (Dreyer & Campbell, 1984; Campbell & Dreyer, 1985).

The purpose of this study was to determine if various plant matrix polysaccharides can differentially affect the feeding behavior of aphids. Pectic fragments (acidic oligosaccharides <2000 daltons), which are breakdown products generated by aphid salivary polysaccharase activity on plant matrix polysaccharides, are found in aphid honeydew (Campbell, 1985). Thus, these compounds are ingested by aphids. There is extensive heterogeneity in the chemical composition and structure of plant matrix polysaccharides between plants and fragments that result from the depolymerization of these polysaccharides can be quite diverse (Bateman & Basham, 1976). During probing, such fragments could serve as gustatory cues to identify host-plants and as a chemical reference for determining the position of stylet tips while searching

for the phloem. To date, no studies have investigated the effects of plant polysaccharides on aphid feeding behavior.

Materials and methods

Aphids. The biotypes of the aphids, used to investigate the effects of plant matrix polysaccharides on aphid feeding behavior, were: *S. graminum* biotype E, *A. pisum* biotype 'Chualar', and *M. persicae* biotype 'Yakima'. Greenbugs were acquired from Dr. S. D. Kindler, USDA, University of Nebraska and maintained on 8 week old sorghum [*Sorghum bicolor* (L.) Moench] cultivar 'IS-809'. Pea aphids were acquired from M. Kinsey, Dept. of Entomology, Univ. of California, Davis and maintained on alfalfa (*Medicago sativa* L.) cultivar 'Caliverde'. Green peach aphids were acquired from Dr. T. E. Mittler, Dept. of Entomology, Univ. of California, Berkeley and maintained on mustard seedlings (*Brassica alba* Rabenh.) cultivar 'Tendergreen'. All colonies of aphids were sustained under similar conditions at $27° ± 5°$ and 12 000 lux of constant light.

Behavioral response bioassays. The feeding responses of the aphids to the plant matrix polysaccharides were evaluated using paired-choice tests. The choice consisted of two vial caps to each of which was added 0.5 ml of a standard holidic diet for aphids (Akey & Beck, 1972). The caps were first sealed with stretched Parafilm® . Next, each of the diets was overlayed with a thin ($143 ± 12$ μm) agar disk. The control consisted of 2% agar whereas the tests consisted of agar incorporated with 1% (w/v) of the test carbohydrate. Agar films were made by pouring 3 ml of a warm agar solution (pH 7.0, 0.1 M P_1 buffer) into a standard petri dish. After cooling, disks were cut from the periphery of the agar with a cork borer. The disks were centered over the diet-filled caps and then covered with a second stretched layer of Parafilm® . One control and one test cap were inserted into the lid of a 28 ml plastic cup in which were placed approx. 50 aphids (apterous virginoparae). The responses of aphids of each species were assayed separately using ten replicates/test carbohydrate/species. The feeding responses of the aphids were evaluated by counting the total number of aphids apparently

probing (viz., stationary) on control vs. test caps after 24 h. Significant differences ($p < 0.05$) between the number of aphids located on and probing the control vs. test caps were analyzed by a paired t-test (Snedecor & Cochran, 1967).

There were no significant differences in the behavioral responses between the species of aphids to the agar controls. Furthermore, the aphids tested showed no significant aggregation behavior when given choice tests in which both caps were overlayed with agar controls. Lastly, staining of salivary sheaths with one percent safranin showed that the stylets of each of the species were capable of penetrating through the agar disk and extending into the artificial diet.

The carbohydrates tested are listed in Table 1. All of the plant polysaccharides and monosaccharides were commercial samples. The acetylated pectins were synthesized according to the methods of Carson & Maclay (1946). The trifluoroacetyl analogue was prepared in a similar manner using trifluoroacetic anhydride.

Electronic recording of probing behavior. Representative probing behavior by aphids was studied by comparing greenbug probing of standard diets overlayed with agar containing either high methoxy pectin (found to be stimulatory; see Results, Table 1) or trifluoroacetyl pectin (highly deterrent, Table 1). The methods for recording aphid probing behavior on the overlays were essentially the same as those used previously (Argandoña et al., 1983) as adapted from the technique introduced by McLean & Kinsey (1964). Aphids were tethered to fine strands (~ 2.0 cm $\times 20$ μm) of gold wire and placed atop the prepared diet-overlays. Aphid probing behavior was recorded for 3 h/overlay with ten replicates each. Recordings of probing behavior were done simultaneously as paired replicates on each type of overlay. The selected probing behavioral parameters that were evaluated are listed in Table 2.

Results

Analytical data demonstrating the differential behaviors elicited by the three species of aphids in response to the various carbohydrates bioassayed are listed in Table 1. None of the monosaccharides induced a negative response by any of the aphids and were, in general, stimulatory. The greenbugs were significantly stimulated by arabinose, xylose, glucose and galacturonic acid. The pea aphids were stimulated by arabinose and glucose whereas the green peach aphids were stimulated by galactose and glucose. Hence, glucose was the only monosaccharide that was a stimulant to all three aphids.

The responses of aphids to the plant matrix polysaccharides were much more variable than were the behaviors observed on the monosaccharides. The behaviors of both greenbugs and pea aphids were significantly affected by all of the plant matrix polysaccharides with only one exception in each case. Greenbugs responded negatively to all of the plant matrix polysaccharides except for high methoxy pectin (stimulatory) and cellulose (no effect). The behavioral responses of pea aphids were significantly deterred by overlays containing low and high methoxy pectin, galactan, and cellulose, but were stimulated by the hemicelluloses, arabinogalactan and xylan. Polygalacturonic acid was the only matrix polysaccharide which had no effect on pea aphid behavior compared to controls. In contrast to the effects on greenbugs and pea aphids, the behavior of green peach aphids was significantly affected by only one plant matrix polysaccharide. Polygalacturonic acid significantly deterred probing by green peach aphids while the remaining matrix polysaccharides elicited no significant departure in probing behavior compared to the controls.

The other plant polysaccharides (i.e., polysaccharides which were either storage carbohydrates or of microbial origin) affected the probing responses of the oligophagous aphids less than green peach aphids. Both starch and amylose significantly deterred probing by green peach aphids (Table 1); neither of these storage polysaccharides affected greenbug or pea aphid probing. However, the reserve polysaccharide of brown algae, laminarin, significantly deterred the probing of all three species of aphids, and the microbial polysaccharide, dextran, had no effect on the probing response of any of the aphids.

The effect of the acetylated pectins on aphid probing behavior varied amongst the aphid species. The acetylated pectins were strongly deterrent to greenbugs but had no effect on pea aphid probing (Table 1). Alternatively, green peach aphid probing

Table 1. Differential behavioral responses (as percent of controls) by aphids to diets overlayed with agar disks incorporated with various mono- or polysaccharides[a].

Carbohydrate	Aphid response		
	S. graminum	*A. pisum*	*M. persicae*
monosaccharide			
arabinose	166.9*	128.3*	107.0
xylose	127.0*	86.0	89.2
galactose	93.2	88.8	145.8*
glucose	134.7*	125.0*	157.0*
galacturonic acid	121.5*	100.0	115.0
plant matrix polysaccharide			
polygalacturonic acid (citrus)	39.4*	101.5	70.6*
low methoxy pectin (3.5% MeO, citrus)	56.2*	26.6*	93.2
high methoxy pectin (60% MeO, citrus)	126.3*	36.9*	113.0
arabinogalactan (larch)	73.3*	177.6*	80.0
xylan (larch)	74.4*	132.0*	86.5
galactan (gum arabic)	50.7*	73.2*	96.7
cellulose	105.7	75.0*	94.4
other polysaccharide			
starch (potato-tuber)	109.0	70.9	62.8*
amylose (potato-tuber)	84.9	99.3	65.9*
laminarin (*Laminaria digitata*)	73.1*	71.0*	60.4*
dextran (*Leuconostoc mesenteroides*)	104.0	90.7	107.0
acetylated pectin			
2, 3-diacetyl pectin	38.4*	83.8	137.0*
trifluoroacetyl pectin	18.9*	87.0	107.0

[a] Paired *t*-tests were performed using numbers of aphids on each choice. Significant differences ($p < 0.05$) are designated with an asterisk (*).

was strongly stimulated by the 2,3-diacetyl pectin whereas the trifluoroacetyl analogue had no effect.

The results of the electronic monitoring of greenbug probing behaviors on trifluoroacetyl pectin (i.e., deterred ingestion of underlying diet) and the high methoxy pectin (i.e., stimulated ingestion) overlays are summarized in Table 2. Although greenbugs probed the trifluoroacetyl pectin overlays, they never ingested the underlying artificial diet. However, greenbugs probing the high methoxy pectin overlays generally initiated ingestion of the underlying artificial diets within an hour and con-

Table 2. Means of different characteristics recorded over a 3 h period for the probing behavior of greenbugs on diets overlayed with polysaccharides that either deterred (trifluoroacetyl pectin) or stimulated (high methoxy pectin) probing (see Table 1).[a]

Probing Parameters	Polysaccharide overlay	
	High methoxy pectin (stimulant)	Trifluoroacetyl pectin (deterrent)
No. of separate probes	14.5	26.7
Mean time of test probes (sec)	81.4	47.3
Total time of ingestion (min)	29.3	0
Total non-probe time (min)	101.9	152.4

[a] All means were significantly different ($p < 0.05$) between the treatments.

tinued for ~0.5 h. Greenbugs made fewer test probes (viz., non-ingestion probes) of longer duration on the high methoxy pectin overlay than on the trifluoroacetyl overlay. On the high methoxy pectin, there were an average of 15 of these test probes lasing ~1.5 min each, whereas on the trifluoroacetyl pectin overlays, there were almost twice as many test probes lasting < 50 s each. Additionally, the non-probe time (viz., stylets not inserted into either the overlay or underlying diet) was significantly longer on the trifluoroacetyl pectin than on the high methoxy pectin.

Discussion

The results show that plant matrix polysaccharides affect the feeding and probing responses of aphids, differentially. Moreover, a reduced ability to discriminate between plant matrix polysaccharides is associated with greater polyphagy in the aphid species studied.

Previous studies have shown that many plant secondary compounds when incorporated into artificial diets or infused into nonhost plants generally affect the feeding behavior of aphids (Dreyer & Jones, 1981). Few studies have found differential responses between species of aphids to these types of plant compounds. Klingauf (1971) found that a dihydrochalcone, phlorizin (a natural product of apple leaves and buds), stimulated the probing behavior of two aphids, *Aphis pomi* (De Geer) and *Rhopalosiphum insertum* (Walk.), which feed on apple. However, phlorizin was a deterrent to *A. pisum* which does not feed on apple. A later study observed that phlorizin actually deterred feeding by *A. pomi*, as well as *M. persicae* (Montgomery & Arn, 1974). In a more extensive study, Dreyer & Jones (1981) compared the feeding deterrency of 31 different phenolics between *S. graminum* and *M. persicae*. They found that phenolics which deterred feeding by greenbugs also deterred green peach aphids and that greenbugs were no more sensitive to these compounds, in general, than green peach aphids. Additionally, phenolics which they tested from a host plant of greenbugs (wheat) did not differentially affect either species. The results of these above studies do not provide evidence as to the definitive role of plant secondary compounds in aphid − host plant coevolution.

On the other hand, the differential probing responses observed between the aphid species in this study to the various polysaccharides assayed supports the idea that this class of biopolymers plays a significant role in aphid-plant interactions. This view is supported by the following observations: (1) Aphids will invariably contact plant matrix polysaccharides in the course of probing a plant, during penetration of cell walls, the middle lamella or the phloem. Contact with plant secondary compounds can be avoided by aphids since aphid stylets may by-pass cell vacuoles in which these compounds are compartmentalized. (2) Aphids have adapted to stylet penetration of plant cell walls by producing salivary polysaccharases having a broad spectrum of depolymerase activities towards plant matrix polysaccharides. (3) Monophagous or oligophagous aphids may show greater gustatory sensitivity in their responses to plant matrix polysaccharides than do polyphagous aphids.

The mode of action of these polysaccharides on aphid probing may be explained by previous studies on aphid probing behavior, physiology and enzymology. Studies of aphid probing behavior show that prior to reaching the phloem there is a repetitive pattern of brief periods (< 2 min) of salivation, imbibition followed by extravasation (Campbell *et al.*, 1982). This pattern can continue for a number of hours, in some cases [e.g., greenbug on sorghum (Montllor *et al.*, 1983)], prior to contact with the phloem. During this pre-phloem contact phase of probing, salivary enzymes having carbohydrase and polysaccharase, polyphenol oxidase and peroxidase activities (Miles, 1972) are discharged from the tips of the stylets. The anatomical and physiological processes by which aphids could discriminate products (viz. probing cues) of salivary enzyme activity are thoroughly outlined by McLean & Kinsey (1985). Briefly, initial gustation of plant constituents does not occur until imbibed fluids contact the precibarial chemosensilla. In conjunction with the precibarial valve, stimulation of motor processes either result in ingestion or extravasation (i.e., egestion). Extravasation could result either from cues that elicit further probing or from substances that are distasteful. Such a distinction between probing cues and ingestion promoters has been noted for planthoppers (Sogawa, 1982). Recently, several C-glycosylflavones were isolated

from rice and found to stimulate stylet probing by 3 planthoppers that are rice pests (Kim *et al.,* 1985). The feeding responses of the aphids towards monosaccharides, in our study, generally resulted in increasing the feeding preference of aphids for the underlying diet. Perhaps monosaccharides or certain oligosaccharides, as breakdown products of salivary enzyme activity on plant matrix polysaccharides, serve as probing cues.

The responses of the aphids to the various plant matrix polysaccharides were generally negative for *S. graminum* and *A. pisum* (Table 1). Alternatively, the generally indifferent responses of green peach aphids towards the plant matrix polysaccharides (Table 1) may signify a reduced sensitivity in the discrimination of breakdown products of these polysaccharides. The broader host plant range of green peach aphids (van Emden *et al.,* 1969) may be explained by this reduced discrimination. The positive response of green peach aphids to the 2,3-diacetyl pectin (which was either neutral or a strong deterrent to the other two aphids) may be a reflection of the fact that acetylated pectins occur in sugar beets (Keenan *et al.,* 1985), a host-plant of green peach aphids (Haniotakis & Lange, 1974). Acetylated pectins do not occur in the host plants of greenbugs (i.e., grasses) or pea aphids (i.e., legumes). The observation that all three species of aphids were deterred by laminarin may result from its unusual structure; essentially a β-1,3 linked glucan, in addition to containing some unusual heteropolysaccharides (e.g., arabinoxylan) (Peat *et al.,* 1958). Since aphids feed on terrestrial plants they are most likely to contact cellulose, a β-1,4 linked glucan.

The numerous, brief probes and lack of ingestion recorded for greenbugs probing on the agar overlays incorporated with trifluoroacetylated pectin mimicked the probing behavior recorded for greenbugs on nonhost plants (cf. 'rice', Table 2; Campbell *et al.,* 1982), whereas probing by greenbugs on the methylated pectin mimicked that of probing on a susceptible host plant. In both cases there were numerous, initial test probes of the agar overlay, but only with methylated pectin (which stimulated feeding in the bioassays) did final penetration into and ingestion of artificial diet occur. This modest difference between the pectins in methylation or acetylation significantly altered the feeding behavior of greenbugs. Different species or

varieties of plants possess matrix polysaccharides whose compositions of neutral sugars, uronic acids, sugar to sugar linkages and degrees of methylation or acetylation differ greatly (Jarvis, 1984). These differences in the structural properties of matrix polysaccharides between plants may be a factor in the adaptation of aphids to a particular host-plant. This adaptation may require an appropriate complement of salivary polysaccharases capable of depolymerizing the matrix polysaccharides of the respective host plant. The breakdown products from these polysaccharides may, in turn, act as specific probing cues; which awaits more definitive chemosensory studies.

Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Acknowledgement

Zusammenfassung

Einfluss verschiedener pflanzlicher Polysaccharide auf das Verhalten von Blattläusen

Drei Blattlausarten, *Schizaphis graminum, Acyrthosiphon pisum* and *Myzus persicae,* zeigten deutliche Unterschiede in ihrem Probeverhalten auf künstlichen Nährmedien, die mit dünnen (<200 μm), verschiedene Mono- und Polysaccharide enthaltenden Agarschichten bedeckt waren.

Die in den Agarschichten enthaltenen Monosaccharide bewirkten bei allen drei Arten eine deutliche Steigerung des Probeverhaltens. Im Gegensatz dazu war der Einfluss der Polysaccharide von Art zu Art verschieden. Sechs von sieben getesteten pflanzlichen Matrixpolysachariden beeinflussten deutlich das Probeverhalten von *Schizaphis graminum* und *Acyrthosiphon pisum.* Im Gegensatz dazu wurde das Probeverhalten von *Myzus persicae* nur von einem der untersuchten Polysaccharide

beeinflusst. Die Zahl der Wirtspflanzen für *Schizaphis graminum* und *Acyrthosiphon pisum* ist relativ klein im Vergleich zu der von *Myzus persicae*. Dies ist möglicherweise darauf zurückzuführen, daß die Fähigkeit, chemosensorisch zwischen pflanzlichen Matrixpolysacchariden zu unterscheiden, bei *Myzus persicae* weniger stark ausgeprägt ist.

Elektronische Registrierung des Probeverhaltens zeigte, daß *Schizaphis graminum* vor der Aufnahme der künstlichen Nährmedien zunächst die darüber liegenden Agarschichten testete. Dies geschah durch Einstechen des Stiletts in die Agarschicht, Absonderung von Speichel, Aufsaugen der verflüssigten Matrix und auschliessendes Wiederausstoßen.

Das Probeverhalten von *Schizaphis graminum* auf Trifluoroacetylpektin, welches eine Aufnahme der darunter liegenden Nährstoffe verhinderte, war vergleichbar zu dem Probeverhalten von *Schizaphis graminum* auf resistenten oder Nichtwirtspflanzen. Andererseits glich das Probeverhalten von *Schizaphis graminum* stark methyliertem Pektin, welche die Nährstoffaufnahme steigerte, dem auf natürlichen Wirtspflanzen. Die Ergebnisse dieser Untersuchung zeigen, daß pflanzliche Matrixpolysaccharide für die Wechselwirkung zwischen Blattläusen und Pflanzen keine chemisch inerten Komponenten darstellen. Der unterschiedliche Einfluss der pflanzlichen Matrixpolysaccharide auf die verschiedenen Blattlausarten zeigt, daß diese pflanzlichen Biopolymere eine entscheidene Rolle bei der Erkennung von Wirtspflanzen durch Blattläuse spielen könnten.

Darüberhinaus zeigt das extrem unterschiedliche Probeverhalten von *Schizaphis graminum* auf Trifluoroacetyl- bzw. stark methyliertem Pektin, daß selbst geringe Änderungen in den chemischen Eigenschaften dieser Komponenten das Probeverhalten von Blattläusen deutlich beeinflussen können.

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